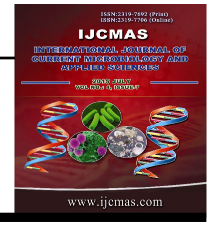

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 4 Number 7 (2015) pp. 624-631**
http://www.ijcmas.com

Original Research Article

Diversity and Density of Coliform Bacteria in River Tunga at Shivamogga city, Karnataka, India

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A B S T R A C T

Keywords

Coliform bacteria, Water, River, Pollution

Water resources are being polluted due to increasing urbanization, modern practices in agriculture and other human activities. The link between human health and environmental pollution is of significant dimension. Studies were conducted with an objective of assessing the coliforms density and dynamics in river Tungawater. River is polluted with domestic and agriculture wastes of Shivamogga city. Water samples were collected from four designated sampling stations of the river. Results revealed that, all the samples from the selected stations contained coliforms bacteria above the safe limits for drinking water standards. Thus river water is unsatisfactory as judged by mean MPN counts of indicator bacteria i.e., total coliforms – 2.22 x 10^7/100 ml. Seasonal variations in coliforms density were recorded with maximum density in summer and monsoon seasons and minimum density in winter seasons.

Introduction

Water contamination by various routes and agents are liable to cause clinically signified outbreaks of intestinal infections in both animals and man. The demonstration of pathogenic bacteria, would obviously constitute the most direct proof of a hazardous impurity; but these pathogens, if present are so scanty that the technical difficulty of their isolation makes the test impractical for ordinary purposes and they are very sensitive even to a slight change of environmental factors and difficult to isolate. Hence, it is impractical to attempt directly to detect the presence of all the different kinds of water borne pathogens, any of which may be present only intermittently. Thus, we rely on tests that reflect the presence of commensal bacteria of intestinal origin such as those of the coliform group, which are more numerous, more easily tested and are the most reliable indicators of fecal pollution (Senior, 1989). Each person discharges 100 to 400 billion coliform organisms/day, in addition to other kinds of bacteria. These coliforms do not themselves constitute a hazard since, they are harmless to man and are in fact useful in destroying organic matter in biological waste treatment processes. But they indicate that faecal matter has entered and that the water is therefore, liable to contamination with more dangerous organisms (Senior, 1989). Presence of coliform organisms is thus taken to indicate possible presence of pathogenic organisms.

Coliform monitoring being rapid, inexpensive easy to perform, proved effective (Leclerc *et al.*, 2000). Coliform was the term first used in 1880's to describe rod-shaped bacteria. Coliform group of bacteria is functionally-related groups belong to a single taxonomic family Enterobacteriaceae and comprises many genera and species. The term coliform organisms refers to gram-negative, rod-shaped bacteria capable of grow in presence of bile salts or other surface-active agents with similar growth-inhibiting properties and able to ferment lactose at 35 $^{\circ}$C–37 $^{\circ}$C with the production of acid, gas and aldehyde within 24-48 hours (Clesceri *et al.*, 1998). They are also oxidase-negative and non-spore-forming and display b-galactosidase activity (Arora, 2003). Traditionally coliform bacteria were regarded as belonging to the genera *Escherichia, Citrobacter*, *Enterobacter* and *Klebsiella.* MacConkey (1909) defined 128 coliform types and this number was increased to 256 by a system developed by Bergey and Deehan (1908). However, as defined by modern taxonomic methods, the group is heterogenous consisting of as many as 32 genera (Leclerc *et al.*, 2001). Some of these can be found in both faeces and the environment (nutrient-rich waters, soil, decaying plant material) as well as in drinking water with relatively high concentration of nutrients and may multiply. Eg. *Serratia fonticola, Rahnella aquatilis, Buttiauxella agrestis.* Coliform bacteria can be indicators for potential pathogens responsible for various waterborne diseases (Hunter *et al.*, 2004) or be pathogens themselves, such as certain strains of *E. coli*. In the present experiment we tested water pools in 96 samples at a stretch of 15 km of Tunga River for abundance and species richness of coliform and other environmental bacteria. We hypothesized that river with more human or animal anthropogenic activities or watershed with agricultural or sewage runoff would have higher bacterial abundance and species richness.

Material and Methods

The present study area covered a stretch of 15 km of the river Tunga near Shivamogga city. Ninety six water samples from Tunga river were collected during April 2010-March 2012 (24 months) from all the four designated stations and were analysed for the levels (MPNs) of generally accepted faecal pollution indicators i.e., total coliforms (TC). Seasonal variations were examined among these indicators during April 2010 to March 2012 as well as the species composition for each group of indicators. The pollution range, maxima, minima, standard deviation of the above parameter has been given along with the detailed discussion of the parameter including ANOVA results. Presumptive coliform count: Single and Double strength MacConkey's broth medium (Hi-media, Ltd.) was prepared by dissolving 4 g and 8 g of MCB in 100 ml of distilled waters separately. Measured amounts were sterilized in test tubes containing a Durham's tube for indicating gas production. With sterile graduated pipettes or with adjustable micropipettes that can hold sterilized microtips, the following amounts of the sampled waters were added.

a. Five 10 ml quantities each to DS (Double Strength) medium.
b. Five 1 ml quantities each to 10 ml SS (Single strength) medium.
c. Five 0.1 ml quantities each to 10 ml SS medium.

Tubes that show acid and sufficient gas to fill the apex covering the concavity of Durham's tube at 24 hours were considered to be presumptive positive, as a result of the growth of coliform Bacilli after incubation at 37 °C. Incubation was sustained from the next session; tubes showing presumptive positive at 48 hours of incubation were accredited to the existing numbers in the concerned station. Sample dilutions were read separately and the positives in A, B and C were serially collated in probability tables to obtain presumptive coliform Bacilli in 100 ml of the sampled station. Tubes showing gas production at 37 °C for 48 hours were confirmed by plating in MacConkey agar plates (MCA) and examined for typical colonies.

Results and Discussion

In the present investigation two-way ANOVA was employed taking test stations (TS_1, TS_2, TS_3 and TS_4) as first independent variable and seasons (summer, rainy and winter) as second independent variable against all parameters considered as dependent variables. The first variable (stations) varied at 4-levels, 2nd variable (seasons) varied at 3-levels to make 4x3 design. This will yield different between the stations, difference between season's interaction between stations and seasons (Lateef *et al.*, 2005). The maximum, minimum and averages of two years in the total Coliforms are represented in table 1. In the current study, no significant difference was noticed between stations and seasons, in the total coliforms and the interaction effect between station and season is also statistically non-significant as all the obtained values failed to reach the significant level. Even though, we find comparative differences among means, these mean differences are statistically insignificant, as there were high individual variations in the data, which is evident from the respective standard deviation (SD) values (Table 2). Presence of coliform organisms in water has been attributed to influxes of allochthonous bacteria from waste discharges and surface water drainage. Total coliform levels were always higher than faecal coliform levels, which is not surprising since total coliforms can originate from non-faecal sources also such as plants and soils (Geldreich, 1974). The above findings and opinions also were corroborated by Goyal *et al.* (1977).

In the present study, high coliform load has been registered during the entire course of study. The MPN of coliform organisms fluctuated from 17×10^1 to 16×10^8 / 100 ml. The higher values of MPN for coliforms were recorded at TS_3 and TS_4, mainly attributed to the domestic sewage and municipal wastes. Though, the values remain statistically significant, due to high individual variations in the data, as per values in the current study the distribution of total coliforms MPN was found bimodal with summer and monsoon maxima. Higher coliform levels occurring during summer months suggest survival and high influx of the coliforms and also increased nutrient influx from decomposition of summer productivity because of high temperature and watershed drainage may also contribute to March-April increase in numbers of both the years. In addition, heavy usage of the river was found during summer for bathing, washing and such other anthropogenic activities, that also would contribute for higher incidence of coliforms. Water quality of Tunga river showed significant deterioration in view of global standards. All most all the samples were contaminated with coliform bacteria, resulting mainly due to anthropogenic activities, especially discharging of domestic and agricultural wastes directly into the river as some recent studies (Sharma *et al.,* 2010; Sadat *et al.,* 2011) revealed that coliform count has positive relation with anthropogenic activities. Present results are in agreement with the findings of Geldrich (1972) who observed an increase in the total coliform count of water bodies due to increased use of animal wastes as manure in the agricultural fields.

In contrast, Goyal *et al.* (1977), Sayler *et al.* (1975) found higher organisms in winter months than in summer. In the present study summer maxima was followed by rainy season. Feachem (1974), Gerba and Schaiberger (1975) have also reported an increase in the number of indicator organisms and Faechem (1974) found peaks of coliforms during on immediately after a storm and other times due to unexplained factors. Occurrence of coliforms in the samples is also confirmed by study conducted by Lateef *et al.* (2003) reporting high coliform counts in the spring water of Kashmir valley. Due to change in agricultural methods, diminishing of livestock farms, intensive farming operation increased concentration of animal wastes results in an increased pollution of rivers and streams (Gelt, 1998). High coliform incidence is influenced by the entry of surface runoff water from nearby areas that carried higher levels of suspended matter and nutrients on their surface. Similar observations were made by Venkateswarlu (1986) in the rivers of Andhra Pradesh. According to Keller (1960) access of soil and organic matter and increase in temperature is responsible for the increase in bacterial numbers in river water after rain and during summer. This is supported by Raj Kumari Sing *et al.* (2001) who carried investigations in Narmada River at Hoshangabad. In Lake Windermere, Taylor (1940) has observed that there is a close relationship between the bacterial content of the water and the rainfall in the drainage area during the preceding week. Subramanyan and Bhaskaran (1948), Voelkar *et al.* (1960) noted a considerable decrease in the number of coliforms in dug wells during cold months.

Further, TS_2 and TS_3 have bathing ghats, where disturbance due to human activities is seen throughout the year. Highest incidence was recorded in TS_4 followed by TS_3, TS_2 and TS_1. Coliform load in river water indicated that bacteria were always and under all conditions remains in water body (Agarwal, 1993). High counts of bacteria signified the organic pollution in river water of Tunga. Extremely high MPN values of Total coliforms i.e., 1600×10^{10} were reported near Opium factory in Ganga water (Shukla Suresh *et al.,* 1992). They also quote the observations of other workers that there is a direct effect of sewage containing faecal matter on the growth and population of coliform bacteria. Agarwal (1993) registered a range of 19-22000/100 ml of TC in river Betwa. Kataria *et al.* (1997) studied river Halali and reported 240 to 2400/100 ml. Doctor *et al.* (1998) recorded MPN between 300 to 1600/100 ml in river Bhadar. Raka *et al.* (1999) registered the faecal pollution in drinking water of Maharashtra and reported the coliform in range of 21–180/100 ml. Koshy Mathew and Vasudevan Nayar (2000) point out that, MPN of Pamba river water was infinite at four of the seven stations considered due to Sabarimala pilgrimage and free flow of sewage, domestic waste and faecal matters into the river at those stations.

Table.1 Sectoral range and mean values of total coliforms (TC/100ml)

Sampling Stations	Mean	Std. Deviation	Std. Error	Minimum	Maximum
TS1	4252.5000	11273.1402	2301.1201	170.00	54000.00
TS2	1464.1667	1317.8504	269.0051	220.00	5400.00
TS3	450541.67	588224.0019	120070.72	13000.00	1600000
TS4	88260000	324878798.22	66315607	940000.0	160000000
Total	22179065	164390697.01	16778055	170.00	160000000

Table.2 Sectoral and seasonal variation of total coliforms (TC/100 ml) with ANOVA results

Seasons	TS_1		TS_2		TS_3		TS_4		Overall	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Summer	$7.39x10^3$	$1.88x10^4$	$8.7x10^2$	$4.15x10^2$	$3.66x10^4$	$2.15x10^4$	$2.36x10^8$	$5.54x10^8$	$5.90x10^7$	$2.83x10^8$
Rainy	$4.85x10^3$	$5.88x10^3$	$1.74x10^3$	$1.76x10^3$	$1.06x10^6$	$6.50x10^5$	$2.64x10^7$	$4.53x10^7$	$6.87x10^6$	$2.44x10^7$
Winter	$5.16x10^2$	$4.58x10^2$	$1.79x10^3$	$1.35x10^3$	$2.53x10^5$	$2.19x10^5$	$2.52x10^6$	$2.75x10^6$	$6.93x10^5$	$1.69x10^6$
Overall	$4.25x10^3$	$1.13x10^4$	$1.46x10^3$	$1.32x10^3$	$4.51x10^5$	$5.88x10^5$	$8.83x10^7$	$3.25x10^8$	$2.21x10^7$	$1.64x10^8$

F for stations (A) = 1.808, P=0.152 (Non-significant); F for seasons (B) = 1.273, P=0.285 (Non-significant); Interaction F (AxB) = 1.281, P=0.275 (Non-significant). Mean values followed by different letters are significantly different from each other.

They also indicate an increased coliform content at the sides of the Pampa River (1600/100 ml) and very low values at the middle part (12/100 ml). They attribute this to the good velocity at middle with no flow near the sides due to sand mining. Shafi *et al.* (2013) observed that, all the samples obtained from the Manasbal Lake of Kashmir were positive with respect to coliform occurrence, though the count was variable ranging between 4 and 460 MPN/100 ml highest proportion of indicator coliforms was found in water samples collected at the site surrounded by residential hamlets in comparison to other three sites. Debashish Mandal and Mukhyopadhyay (1991) also report extremely high MPN ranging from $10x10^2$ to $9x10^6$/100 ml in river Ganga. According to them coliform bacteria are more abundant in certain sewage channels and bathing ghats. Musaddiq Mohammed (2000) recorded a maximum MPN of 900 in Morna River and suggests health education as an important weapon in creating desire for high standard of life. Rajkumar Singh *et al.* (2001) noted MPN index up to 9200/100 ml in Narmada River signifying the organic pollution in the water body due to the human activities. Asthana and Sing (1993) observed extremely high MPN values of $21x10^3$ to $40x10^3$ in Gomati River with faecal coliform accounting for nearly 60 to 80% of the total coliform. Praveen Jain and Sanjay Telang (1996) have shown an MPN of 118 in Parbathy River at Sehore (M.P) and conclude that river water has minimal bacterial load. Dutka (1973), Evison and James (1973), Burrel and Roland (1979), Hazen (1988), Ramteke *et al.* (1992) advocate that, total coliform count performed by the most probable number (MPN) method is commonly used indicator of potability despite the drawbacks that have been well documented.

It is concluded from the present results that, existence of both non-faecal bacteria that fit the definitions of coliform bacteria and of lactose-negative coliform bacteria limits the applicability of this group as an indicator of faecal pollution. Drinking directly from river without filtration should be avoided at all times. Many organisms present in river are very sensitive to ecosystem. Coliform bacteria should not be detectable in treated water supplied and if found, suggest inadequate treatment, post treatment contamination or excessive nutrients. Coliform test can therefore be used as indicator for both treatment efficiency and integrity of distribution system. Although coliform organisms may not always be directly related to the presence of faecal contamination or pathogens in drinking water, coliform test is still useful for monitoring the microbial quality of treated piped water supplies. If there is any doubt, especially when coliform organisms are found in the absence of thermo tolerant coliforms and *E. coli*, identification to the species level or analyses for other indicator organisms may be undertaken to investigate the nature of contamination. Sanitary inspections will also be needed.

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